# WEST Search History

DATE: Monday, November 24, 2003

| Set Name side by side | Query | Hit Count | Set Name result set |
|---|---|---|---|
| | *DB=USPT,PGPB,EPAB,DWPI,TDBD; THES=ASSIGNEE; PLUR=YES; OP=ADJ* | | |
| L3 | troponin same (isolat$ or purif$ or chromotograp$) and sulfhydryl and sulfitolyz$ | 4 | L3 |
| L2 | troponin same (isolat$ or purif$ or chromotograp$) and sulfhydryl | 15 | L2 |
| L1 | troponin same (isolat$ or purif$ or chromotograp$) same sulfhydryl | 5 | L1 |

END OF SEARCH HISTORY

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**Search Results** - Record(s) 1 through 15 of 15 returned.

1. Document ID: US 20030166062 A1

L2: Entry 1 of 15 File: PGPB Sep 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030166062
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030166062 A1

TITLE: Methods and compositions for production of recombinant peptides

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Gonzalez-Villasenor, Lucia Irene | Baltimore | MD | US | |

US-CL-CURRENT: 435/69.1; 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

2. Document ID: US 20030138907 A1

L2: Entry 2 of 15 File: PGPB Jul 24, 2003

PGPUB-DOCUMENT-NUMBER: 20030138907
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030138907 A1

TITLE: Purification of human troponin I

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Conn, Gregory | Cary | NC | US | |
| Reardon, Brian | Seattle | WA | US | |
| Zeng, Xianfang | Northborough | MA | US | |
| Zhang, Chenming | Blacksburg | VA | US | |

US-CL-CURRENT: 435/69.1; 435/252.33, 435/320.1, 530/350, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

3. Document ID: US 20030130224 A1

L2: Entry 3 of 15 File: PGPB Jul 10, 2003

PGPUB-DOCUMENT-NUMBER: 20030130224

PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030130224 A1

TITLE: Expression of zeta negative and zeta positive nucleic acids using a dystrophin gene

PUBLICATION-DATE: July 10, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Monahan, Sean D. | Madison | WI | US | |
| Wolff, Jon A. | Madison | WI | US | |
| Slattum, Paul M. | Madison | WI | US | |
| Hagstrom, James E. | Madison | WI | US | |
| Budker, Vladimir G. | Madison | WI | US | |
| Rozema, David B. | Madison | WI | US | |

US-CL-CURRENT: 514/44; 602/13

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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4. Document ID: US 20030105017 A1

L2: Entry 4 of 15 File: PGPB Jun 5, 2003

PGPUB-DOCUMENT-NUMBER: 20030105017
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030105017 A1

TITLE: Purification of human Troponin I

PUBLICATION-DATE: June 5, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Conn, Gregory | Cary | NC | US | |
| Reardon, Brian | Seattle | WA | US | |
| Zeng, Xianfang | Northborough | MA | US | |
| Zhang, Chenming | Blacksburg | VA | US | |

US-CL-CURRENT: 514/12; 435/69.1, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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5. Document ID: US 20020064835 A1

L2: Entry 5 of 15 File: PGPB May 30, 2002

PGPUB-DOCUMENT-NUMBER: 20020064835
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020064835 A1

TITLE: Purification of human troponin I

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Conn, Gregory | Cary | NC | US | |
| Reardon, Brian | Seattle | WA | US | |
| Zeng, Xianfang | Northborough | MA | US | |
| Zhang, Chenming | Blacksburg | VA | US | |

US-CL-CURRENT: 435/71.2; 514/2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments  KWIC | Draw. Desc | Image

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## 6. Document ID: US 20020055145 A1

L2: Entry 6 of 15 File: PGPB May 9, 2002

PGPUB-DOCUMENT-NUMBER: 20020055145
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020055145 A1

TITLE: Purification of human troponin I

PUBLICATION-DATE: May 9, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Conn, Gregory | Cary | NC | US | |
| Reardon, Brian | Seattle | WA | US | |
| Zeng, Xianfang | Northborough | MA | US | |
| Zhang, Chenming | Blacksburg | VA | US | |

US-CL-CURRENT: 435/69.1; 530/417

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments  KWIC | Draw. Desc | Image

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## 7. Document ID: US 6589936 B1

L2: Entry 7 of 15 File: USPT Jul 8, 2003

US-PAT-NO: 6589936
DOCUMENT-IDENTIFIER: US 6589936 B1

TITLE: Pharmaceutical compositions comprising recombinant troponin subunits

DATE-ISSUED: July 8, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Thorn; Richard M. | North Easton | MA | | |
| Lanser; Marc E. | Dover | MA | | |
| Moses; Marsha A. | Brookline | MA | | |
| Wiederschain; Dmitri G. | Brookline | MA | | |

US-CL-CURRENT: 514/12; 435/69.1, 435/70.1, 514/2, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments  KWIC | Draw Desc | Image

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8. Document ID: US 6586401 B1

L2: Entry 8 of 15 File: USPT Jul 1, 2003

US-PAT-NO: 6586401
DOCUMENT-IDENTIFIER: US 6586401 B1

TITLE: Troponin subunit I fragment and homologs thereof

DATE-ISSUED: July 1, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Thorn; Richard M. | North Easton | MA | | |
| Lanser; Marc E. | Dover | MA | | |
| Moses; Marsha A. | Brookline | MA | | |
| Wiederschain; Dmitri G. | Drighton | MA | | |

US-CL-CURRENT: 514/13; 530/326

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments  KWIC | Draw Desc | Image

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9. Document ID: US 6465431 B1

L2: Entry 9 of 15 File: USPT Oct 15, 2002

US-PAT-NO: 6465431
DOCUMENT-IDENTIFIER: US 6465431 B1

TITLE: Pharmaceutical compositions comprising troponin subunits, fragments and homologs thereof and methods of their use to inhibit angiogenesis

DATE-ISSUED: October 15, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Thorn; Richard M. | North Easton | MA | | |
| Lanser; Marc E. | Dover | MA | | |
| Moses; Marsha A. | Brookline | MA | | |
| Wiederschain; Dmitri G. | Brookline | MA | | |

US-CL-CURRENT: 514/16; 530/328

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments  KWIC | Draw Desc | Image

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10. Document ID: US 6403558 B1

L2: Entry 10 of 15 File: USPT Jun 11, 2002

US-PAT-NO: 6403558
DOCUMENT-IDENTIFIER: US 6403558 B1

TITLE: Pharmaceutical compositions comprising troponin subunits, fragments and analogs thereof and methods of their use to inhibit angiogenesis

DATE-ISSUED: June 11, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Moses; Marsha A. | Brookline | MA | | |
| Langer; Robert S. | Newton | MA | | |
| Wiederschain; Dimitri G. | Brookline | MA | | |
| Wu; Inmin | Boston | MA | | |
| Sytkowski; Arthur | Arlington | MA | | |

US-CL-CURRENT: 514/12; 514/21, 530/324

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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11. Document ID: US 6025331 A

L2: Entry 11 of 15 File: USPT Feb 15, 2000

US-PAT-NO: 6025331
DOCUMENT-IDENTIFIER: US 6025331 A

TITLE: Pharmaceutical compositions comprising troponin subunits, fragments and analogs thereof and methods of their use to inhibit angiogenesis

DATE-ISSUED: February 15, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Moses; Marsha A. | Brookline | MA | | |
| Langer; Robert S. | Newton | MA | | |
| Wiederschain; Dimitri G. | Brookline | MA | | |
| Wu; Inmin | Boston | MA | | |
| Sytkowski; Arthur | Arlington | MA | | |

US-CL-CURRENT: 514/12; 514/2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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12. Document ID: US 5948771 A

L2: Entry 12 of 15 File: USPT Sep 7, 1999

US-PAT-NO: 5948771
DOCUMENT-IDENTIFIER: US 5948771 A

TITLE: Method for treating heart failure using tetrapyrroles and metallotetrapyrroles

DATE-ISSUED: September 7, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Danziger; Robert S. | New York | NY | | |

US-CL-CURRENT: 514/185; 540/145

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments  KWIC | Draw Desc | Image

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13. Document ID: US 5846738 A

L2: Entry 13 of 15 File: USPT Dec 8, 1998

US-PAT-NO: 5846738
DOCUMENT-IDENTIFIER: US 5846738 A

TITLE: Synthetic standard for immunoassays

DATE-ISSUED: December 8, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Seidel; Christoph | Weilheim | | | DE |
| Bialk; Peter | Eberfing | | | DE |
| Von der Eltz; Herbert | Weilheim | | | DE |

US-CL-CURRENT: 435/7.1; 435/13, 435/5, 435/69.3, 435/7.9, 435/7.92, 435/961, 435/967, 435/973, 436/517, 436/518, 436/536 , 436/8, 530/324, 530/325, 530/326, 530/327, 530/328, 530/329, 530/350, 530/806

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments  KWIC | Draw Desc | Image

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14. Document ID: US 5837680 A

L2: Entry 14 of 15 File: USPT Nov 17, 1998

US-PAT-NO: 5837680
DOCUMENT-IDENTIFIER: US 5837680 A

TITLE: Pharmaceutical compositions comprising troponin subunits, fragments and analogs thereof and methods of their use to inhibit angiogenesis

DATE-ISSUED: November 17, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Moses; Marsha A. | Brookline | MA | | |
| Langer; Robert S. | Newton | MA | | |
| Wiederschain; Dimitri G. | Brookline | MA | | |
| Wu; Inmin | Boston | MA | | |
| Sytkowski; Arthur | Arlington | MA | | |

US-CL-CURRENT: 514/12; 514/21, 530/324

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments  KWIC | Draw Desc | Image

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15. Document ID: US 20030138907 A1 WO 200204512 A2 AU 200173348 A US 20020055145 A1 US 20020064835 A1 US 20030105017 A1

L2: Entry 15 of 15 File: DWPI Jul 24, 2003

DERWENT-ACC-NO: 2002-154921
DERWENT-WEEK: 200352
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TITLE: Purifying troponin I comprises subjecting troponin I to chromatography on anion exchanger after reversibly protecting the free sulfhydryl groups

INVENTOR: CONN, G; REARDON, B ; ZENG, X ; ZHANG, C

PRIORITY-DATA: 2000US-217069P (July 10, 2000), 2001US-0903398 (July 10, 2001), 2001US-0998619 (November 30, 2001), 2002US-0255244 (September 26, 2002), 2002US-0287118 (November 4, 2002)

PATENT-FAMILY:

| PUB-NO | PUB-DATE | LANGUAGE | PAGES | MAIN-IPC |
|---|---|---|---|---|
| US 20030138907 A1 | July 24, 2003 | | 000 | C12P021/02 |
| WO 200204512 A2 | January 17, 2002 | E | 028 | C07K014/47 |
| AU 200173348 A | January 21, 2002 | | 000 | C07K014/47 |
| US 20020055145 A1 | May 9, 2002 | | 000 | C12P021/02 |
| US 20020064835 A1 | May 30, 2002 | | 000 | C12P021/04 |
| US 20030105017 A1 | June 5, 2003 | | 000 | A61K038/17 |

INT-CL (IPC): A61 K 38/00; A61 K 38/17; C07 H 21/04; C07 K 1/16; C07 K 14/47; C12 N 1/21; C12 P 21/02; C12 P 21/04; C12 P 21/06

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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| Term | Documents |
|---|---|
| TROPONIN | 1356 |
| TROPONINS | 102 |
| SULFHYDRYL | 14036 |
| SULFHYDRYLS | 958 |
| ISOLAT$ | 0 |
| ISOLAT | 37 |
| ISOLATA | 2 |
| ISOLATABILITY | 31 |
| ISOLATABLE | 2232 |
| ISOLATABLE-TYPE | 1 |
| (TROPONIN SAME (ISOLAT$ OR PURIF$ OR CHROMOTOGRAP$) AND SULFHYDRYL).USPT,PGPB,EPAB,DWPI,TDBD. | 15 |

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=> s troponin (s) (isolat? or purif? or chromotograp?) and sulfhydryl and sulfitolyz?
L1 0 FILE ADISCTI
L2 0 FILE ADISINSIGHT
L3 0 FILE ADISNEWS
L4 0 FILE AGRICOLA
L5 0 FILE ANABSTR
L6 0 FILE AQUASCI
L7 0 FILE BIOBUSINESS
L8 0 FILE BIOCOMMERCE
L9 0 FILE BIOSIS
L10 1 FILE BIOTECHDS
L11 0 FILE BIOTECHNO
L12 0 FILE CABA
L13 0 FILE CANCERLIT
L14 1 FILE CAPLUS
L15 0 FILE CEABA-VTB
L16 0 FILE CEN
L17 0 FILE CIN
L18 0 FILE CONFSCI
L19 0 FILE CROPB
L20 0 FILE CROPU
L21 0 FILE DISSABS
L22 0 FILE DGENE
L23 0 FILE DRUGB
L24 0 FILE DRUGLAUNCH
L25 0 FILE DRUGMONOG2
L26 0 FILE DRUGNL
L27 0 FILE DRUGU
L28 0 FILE DRUGUPDATES
L29 0 FILE EMBAL
L30 0 FILE EMBASE
L31 0 FILE ESBIOBASE
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'TROPONIN (S) '
L32 0 FILE FEDRIP
L33 0 FILE FOMAD
L34 0 FILE FOREGE
L35 0 FILE FROSTI
L36 0 FILE FSTA
L37 0 FILE GENBANK
L38 0 FILE HEALSAFE
L39 4 FILE IFIPAT
L40 0 FILE JICST-EPLUS
L41 0 FILE KOSMET
L42 0 FILE LIFESCI
L43 0 FILE MEDICONF
L44 0 FILE MEDLINE
L45 0 FILE NIOSHTIC
L46 0 FILE NTIS
L47 0 FILE NUTRACEUT
L48 0 FILE OCEAN
L49 0 FILE PASCAL
L50 0 FILE PCTGEN
L51 0 FILE PHAR
L52 0 FILE PHARMAML

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L55           0 FILE PROMT
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L61           0 FILE USPAT2
L62           0 FILE VETB
L63           0 FILE VETU
L64           1 FILE WPIDS
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L79           0 FILE CBNB
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L81           0 FILE COPPERLIT
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L83           0 FILE ENCOMPLIT2
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L85           0 FILE INSPHYS
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L90           0 FILE RAPRA
L91           0 FILE RUSSCI
L92           0 FILE STANDARDS
L93           0 FILE TULSA
L94           0 FILE TULSA2
L95           0 FILE USAN
L96           0 FILE WELDASEARCH
L97           0 FILE WSCA

TOTAL FOR ALL FILES
L98          11 TROPONIN (S) (ISOLAT? OR PURIF? OR CHROMOTOGRAP?) AND SULFHYDRYL
                AND SULFITOLYZ?

=> dup rem l98
DUPLICATE IS NOT AVAILABLE IN 'ADISINSIGHT, ADISNEWS, BIOCOMMERCE, DGENE,
DRUGLAUNCH, DRUGMONOG2, DRUGUPDATES, FEDRIP, FOREGE, GENBANK, KOSMET,
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PROCESSING COMPLETED FOR L98
L99           5 DUP REM L98 (6 DUPLICATES REMOVED)

=> d l99 1-5 ibib abs

L99  ANSWER 1 OF 5  IFIPAT  COPYRIGHT 2003 IFI on STN  DUPLICATE 1
AN                      10394487  IFIPAT;IFIUDB;IFICDB
TITLE:                  PURIFICATION OF HUMAN TROPONIN I
INVENTOR(S):            Conn; Gregory, Cary, NC, US
```

Reardon; Brian, Seattle, WA, US
Zeng; Xianfang, Northborough, MA, US
Zhang; Chenming, Blacksburg, VA, US
PATENT ASSIGNEE(S): Unassigned
AGENT: INTERVET INC, 405 STATE STREET, PO BOX 318, MILLSBORO, DE, 19966, US

| | NUMBER | PK | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 2003138907 | A1 | 20030724 |
| APPLICATION INFORMATION: | US 2002-287118 | | 20021104 |

| | APPLN. NUMBER | DATE | GRANTED PATENT NO. OR STATUS |
|---|---|---|---|
| CONTINUATION OF: | US 2001-903398 | 20010710 | |
| CONTINUATION OF: | US 2001-998619 | 20011130 | |

| | NUMBER | DATE | |
|---|---|---|---|
| PRIORITY APPLN. INFO.: | US 2000-217069P | 20000710 | (Provisional) |
| FAMILY INFORMATION: | US 2003138907 | 20030724 | |

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
NUMBER OF CLAIMS: 20 11 Figure(s).
DESCRIPTION OF FIGURES:
FIGS. 1A and 1B. The chemical structure of modified cysteine. A. Conversion of cysteine to S-sulfocysteine by reaction with sodium tetrathionate and reversal by exogenous thiols. B. The cleavage of disulfide bonds by sodium sulfite to form the Ssulfo derivative.
FIG. 2. Preparation and washing of TnI-containing inclusion bodies.
FIG. 3. Summary of rTroponin-I preparation.
FIG. 4. Q-Sepharose FF chromatography Troponin I. Buffer A: 6 M urea, 25 mM Tris-HCl, pH 7.5, 100 mM; Buffer B: 6M urea, 25 mM Tris-HCl, pH 7.5, 2M NaCl; Gradient: Step; 0% B for the flowthrough and 100% B for the strip; and Flow rate: 150 ml/min.
FIG. 5. 300 ml Q-sepharose FF chromatography. Buffer A: 6M urea, 25 mM Tris-HCl, pH 7.5, 100 mM; Buffer B: 6M urea, 25 mM TrisHCl, pH 7.5, 2M NaCl; Gradient: Step; 4% B for elution and 50% B for strip; and Flow rate: 20 ml/min.
FIG. 6. SDS-PAGE analysis troponin lot after anion exchange steps no. 1 and no. 2 in 16% tris-glycine gel, under nonreducing conditions. A-H refer to lanes in the SDS-PAGE gel. A. **Sulfitolyzed** troponin Lot 3L4 standard; B. solubilized inclusion bodies; C. **sulfitolyzed** inclusion bodies (AEX No. 1 load); D. anion exchange no. 1 flowthrough; E. anion exchange no. 1 salt eulate; F. anion exchange no. 2 load; G. anion exchange no. 2 flowthrough; and, H. anion exchange no. 2 100 mM NaCl eluate.
FIG. 7. Toyopearl 650M (phenyl) HIC chromatograph. Buffer A: 6M urea, 25 mM Tris-HCl, pH 7.5, 1M (NH4)2SO4; Buffer B: 6M urea, 25 mM Tris-HCl, pH 7.5; Gradient: Step; 100% B for the flowthrough and 0% B for strip; and Flow rate: 10 ml/min.
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FIG. 9. Quantitation of rTnI on Zorbax C3.
FIG. 10. Troponin I LysC mapping.
FIG. 11. SDS-PAGE analysis of **sulfitolyzed** troponin reduction with dithiothreitol for 45 mins. at ambient temperature. One mg/ ml TnI in 6M urea, 25 mM tris, 0.15M NaCl pH 7.5, run on a 16% tris-glycine gel.

AB The invention is directed to methods for **purifying** **Troponin** I, particularly recombinant **Troponin** I

produced in a bacterial expression system. Recombinant **Troponin** I can be advantageously **purified** after reversibly protecting the free **sulfhydryl** groups, e.g., by forming sulfates. In a specific example, Tropnin I reacted with sodium tetrafhionate yields **sulfitolyzed** Tropnin I, which was **purified** by chromatography on an anion exchanger, followed by hydrophobic interaction chromatography. Facile deprotection of the **sulfhydryl** groups yields a highly **purified** product ready for refolding.

CLMN 20 11 Figure(s).

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L99 ANSWER 2 OF 5 IFIPAT COPYRIGHT 2003 IFI on STN DUPLICATE 2

| | | | |
|---|---|---|---|
| AN | 10360600 IFIPAT;IFIUDB;IFICDB | | |
| TITLE: | **PURIFICATION** OF HUMAN **TROPONIN** I | | |
| INVENTOR(S): | Conn; Gregory, Cary, NC, US<br>Reardon; Brian, Seattle, WA, US<br>Zeng; Xianfang, Northborough, MA, US<br>Zhang; Chenming, Blacksburg, VA, US | | |
| PATENT ASSIGNEE(S): | Unassigned | | |
| AGENT: | INTERVET INC, 405 STATE STREET, PO BOX 318, MILLSBORO, DE, 19966, US | | |
| | NUMBER | PK | DATE |
| PATENT INFORMATION: | US 2003105017 | A1 | 20030605 |
| APPLICATION INFORMATION: | US 2002-255244 | | 20020926 |

GRANTED PATENT NO.

| | APPLN. NUMBER | DATE | OR STATUS |
|---|---|---|---|
| DIVISION OF: | US 2001-903398 | 20010710 | |
| FAMILY INFORMATION: | US 2003105017 | 20030605 | |

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
NUMBER OF CLAIMS: 20 11 Figure(s).
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AB The invention is directed to methods for **purifying** **Troponin** I, particularly recombinant Tropnin I produced in a bacterial expression system. Recombinant Tropnin I can be advantageously **purified** after reversibly protecting the free **sulfhydryl** groups, e.g., by forming sulfates. In a specific example, Tropnin I reacted with sodium tetrafhionate yielded **sulfitolyzed** Tropnin I, which was **purified** by chromatography on an anion exchanger, followed by hydrophobic interaction chromatography. Facile deprotection of the **sulfhydryl** groups yields a highly **purified** product ready for refolding.

CLMN 20 11 Figure(s).

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FIG. 5. 300 ml Q-sepharose FF chromatography. Buffer A: 6M urea, 25 mM Tris-HCl, pH 7.5, 100 mM; Buffer B: 6M urea, 25 mM TrisHCl, pH 7.5, 2M NaCl; Gradient: Step, 4% B for elution and 50% B for strip; and Flow rate: 20 ml/min.

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L99 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-08599 BIOTECHDS
TITLE: **Purifying troponin** I comprises subjecting **troponin** I to chromatography on anion exchanger after reversibly protecting the free **sulfhydryl** groups; recombinant production in Escherichia coli and application in e.g. cancer therapy
AUTHOR: CONN G; REARDON B; ZENG X; ZHANG C
PATENT ASSIGNEE: DIOSYNTH RTP INC
PATENT INFO: WO 2002004512 17 Jan 2002
APPLICATION INFO: WO 2000-US21817 10 Jul 2000
PRIORITY INFO: US 2000-217069 10 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-154921 [20]
AN 2002-08599 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Preparing **troponin** I, comprising protecting free **sulfhydryl** groups of **troponin** I under reducing conditions, and **troponin** I is then **purified** by subjecting **troponin** I comprising **sulfhydryl** protecting groups to chromatography, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for **troponin** I comprising **sulfhydryl** protecting groups.

BIOTECHNOLOGY - Preferred Method: The recombinant **troponin** I is expressed in a bacterial expression system, preferably an Escherichia coli expression system. The free **sulfhydryl** groups are protected by **sulfitolyzation** which comprises reacting reduced recombinant **troponin** I with sodium tetrathionate. **Troponin** I is **purified** by chromatography under non-reducing conditions and the **sulfhydryl** groups are deprotected from the **purified troponin** I. The chromatographic support is an anion exchange column, optionally followed by hydrophobic interaction chromatography. **Troponin** I is

denatured and the **sulfhydryl** protecting groups are sulfates.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Inhibitor of angiogenesis. No supporting data is given.

USE - The method is useful for **purifying troponin** I, particularly recombinant **troponin** I. The highly **purified troponin** I, preferably in a refolded state is useful for antibody generation, as a control or standard immunoassay reagent, or to inhibit angiogenesis important in treating various cancers.

ADVANTAGE - Protection of **sulfhydryl** groups during **troponin** I preparation eliminates the costly need for maintaining non-reducing conditions throughout protein preparation, **purification** and storage, and need for reducing agents. The **sulfhydryl**-protected **troponin** does not form intrachain or interchain disulfide crosslinks. Overall yield of **troponin** from the multi-step **purification** was greater than 50% at purity levels of greater than 95%. Deprotection of the **sulfhydryl** groups yields a highly **purified** product ready for refolding.

EXAMPLE - Human skeletal **troponin** I (TnI) expressed in Escherichia coli was **isolated** from lysed cells in inclusion bodies. 10 g of TnI-containing inclusion bodies were solubilized and protein **sulfhydryls** were **sulfitolyzed** using 6 M urea (200 ml), Tris (25 mM), sodium sulfite (10 mg/ml), sodium tetrathionate (5 mg/ml) pH 7.5 at ambient temperature for 6 hours in the dark. The solubilized material was filtered over a 0.2 micro membrane prior to subsequent processing. **Sulfitolyzed** recombinant human TnI was **purified** by a five step process. Solubilized, **sulfitolyzed** TnI-containing inclusion bodies (200 ml) were loaded onto a 3 l volume Q-sepharose FF column pre-equilibrated in 6 M urea, 25 mM Tris, 0.1 M NaCl pH 7.5 at 150 ml/min. The **purified** TnI was collected in the column flowthrough. The recovered TnI was concentrated. This material was loaded onto a 300 ml volume Q-sepharose FF column pre-equilibrated in 6M urea, 25 mM Tris, pH 7.5 at 20 ml/minute. The bound TnI was eluted from the column by a step wash with 6 M urea, 25 mM Tris, 80 mM NaCl pH 7.5. This eluted **troponin** (500 ml) was loaded onto a 60 ml column of Toyopearl 650 M phenyl HIC resin after addition of ammonium sulfate to a final concentration of 1 M. The column was pre-equilibrated with 6 M urea, 25 mM Tris, 1M ammonium sulfate pH 7.5. The **purified troponin** was collected as the unbound flowthrough from this column, concentrated 2.5-fold and buffer exchanged for storage by UF/DF. **Purified** TnI was stored frozen at -70 degrees C. Protein purity was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and reverse phase chromatography and protein identity was confirmed by peptide mapping with peptide mass and fragmentation analysis. Yield determinations for each step were determined by quantitative reverse phase chromatography. Residual DNA levels, measured by DNA threshold, were less than or equal to 12 pg DNA/mg protein. Endotoxin testing of final product by Limulus Amoebocyte Lysate (LAL) (gel-clot) indicated less than or equal to 3 EU/mg protein. (28 pages)

L99 ANSWER 4 OF 5 IFIPAT COPYRIGHT 2003 IFI on STN DUPLICATE 4

AN 10121228 IFIPAT;IFIUDB;IFICDB

TITLE: **PURIFICATION** OF HUMAN **TROPONIN** I; GENERATING MUSCLE PROTEIN; OBTAIN SAMPLE, INCUBATE UNDER REDUCING ENVIRONMENT, RECOVER MUSCLE PROTEIN

INVENTOR(S): Conn; Gregory, Cary, NC, US
Reardon; Brian, Seattle, WA, US
Zeng; Xianfang, Northborough, MA, US
Zhang; Chenming, Blacksburg, VA, US

PATENT ASSIGNEE(S): Diosynth RTP, Inc.

AGENT: DARBY & DARBY P.C., 805 Third Avenue, New York, NY, 10022, US

| | NUMBER | PK | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 2002064835 | A1 | 20020530 |
| APPLICATION INFORMATION: | US 2001-903398 | | 20010710 |

| | NUMBER | DATE |
|---|---|---|
| PRIORITY APPLN. INFO.: | US 2000-217069P | 20000710 (Provisional) |
| FAMILY INFORMATION: | US 2002064835 | 20020530 |
| DOCUMENT TYPE: | Utility<br>Patent Application - First Publication | |
| FILE SEGMENT: | CHEMICAL<br>APPLICATION | |
| NUMBER OF CLAIMS: | 20 11 Figure(s). | |

DESCRIPTION OF FIGURES:

FIGS. 1A and 1B. A. Proposed reaction for oxidative sulfitolysis. B. Cleavage of disulfide bond by sodium sulfite to form the Ssulfo derivative.

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CLMN 20 11 Figure(s).

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| AN | 10111538 IFIPAT;IFIUDB;IFICDB |
| TITLE: | **PURIFICATION** OF HUMAN **TROPONIN** I; **ISOLATING** PREFERENTIAL POLYPEPTIDE; OBTAIN SAMPLE, INCUBATE WITH ION EXCHANGE RESIN, ELUTE, RECOVER POLYPEPTIDE |
| INVENTOR(S): | Conn; Gregory, Cary, NC, US<br>Reardon; Brian, Seattle, WA, US<br>Zeng; Xianfang, Northborough, MA, US<br>Zhang; Chenming, Blacksburg, VA, US |
| PATENT ASSIGNEE(S): | Diosynth RTP, Inc. |
| AGENT: | DARBY & DARBY P.C., 805 Third Avenue, New York, NY, 10022, US |

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| FAMILY INFORMATION: | US 2002055145 | 20020509 | |
| DOCUMENT TYPE: | Utility | | |

Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
NUMBER OF CLAIMS: 20 11 Figure(s).
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